b.) Amendments to the Specification

Please enter the attached "Sequence Listing" into the application.

In addition, please enter the following amendments to the Specification:

[p. 7, last para.] The *msp1α* gene was cloned by PCR from the Oklahoma isolate of *A. marginale* derived from infected erythrocytes. DNA was extracted from 1 ml stored blood samples containing infected bovine erythrocytes collected during high parasitemia employing 250 μL Tri Reagent (Sigma) and following manufacturer's recommendations. Extracted DNA was resuspended in 100 μL water. The *msp1α* gene was amplified from 1 μL DNA by PCR using 10 pmol of each primer MSP1aP: 5'GCATTACAACGCAACGCTTGAG3' (SEQ. ID NO: 1) and MSP1a3: 5'GCTTTACGCCGCCGCCTGCGCC3' (SEQ. ID NO: 2) in a 50-μL volume PCR employing the Access RT-PCR system (Promega). Reactions were performed in an automated DNA thermal cycler (Eppendorf) for 35 cycles. After an initial denaturation step of 30 sec at 94°C, each cycle consisted of a denaturing step of 30 sec at 94°C and an annealing-extension step of 2.5 min at 68°C. The program ended by storing the reactions at 4°C. PCR products were electrophoresed on 1% agarose gels to check the size of amplified fragments. The amplified fragments were resin purified from PCR reactions (Wizard Promega) and cloned into pGEM-T vector (Promega) for sequencing both strands (Core Sequencing Facility, Department of Biochemistry and Molecular Biology, Noble Research Center, Oklahoma State University).

[p. 8, first full para.] For high level expression of MSP1a, *msp1α* coding region was amplified from per1 (*msp1α* in pGEM-T vector) plasmid DNA by PCR using the primers

5'CCGCTCGAGATGTTAGCGGAGTATGTGTCC3' (SEQ. ID NO: 3) and 5'GAAGATCTCGCCGCCGCCTGCGCC3' (SEQ. ID NO: 4). The *msp1α* amplification product was digested with *Xho*I and *Bgl*II and inserted into the cloning site of pFLAG-CTC expression vector (Sigma). Recombinant plasmid was named pFLC1a. In this construct, the inserted gene is under the control of the inducible *tac* promoter and yield full-length MSP1a polypeptide, with a C-terminal fusion of a FLAG marker octapeptide. The fidelity and orientation of the construct was verified by sequencing. For expression of MSP1a recombinant polypeptides, pFLC1a expression plasmid was transformed into *E. coli* K-12 (strain JM109). Transformed *E. coli* strains were inoculated in LB containing 50 μg/ml Ampicillin and 0.4% glucose. Cultures were grown at 37°C to OD_{600nm}=0.4. IPTG was then added to 0.5 mM final concentration, and incubation continued during 4 h, for induction MSP1a expression. Cells were collected by centrifugation and membranes extracted after sonication and centrifugation. MSP1b was cloned, expressed and purified in a similar way. Doses of 5 ml containing 100 μg recombinant antigens were used for vaccination in subsequent studies.

[p. 11, first para.] 4. *Immunizations*. All cattle were immunized 3 times by subcutaneous injection of the antigen at weeks 1, 4 and 6. Each antigen dose was 5 ml in volume and contained an antigen in the adjuvant, ~~Xtend~~XTEND® III (Grand Laboratories, Larchwood, IA). All cattle were challenge-exposed 10 weeks after the last immunization with 1 x 10^7 *A. marginale* infected erythrocytes collected from a calf experimentally infected with the Oklahoma isolate of *A. marginale*. Blood of the immunized and control cattle was monitored for infection with *A. marginale* by microscopic examination of blood smears and hematology was done daily after the onset of infection. Parameters evaluated in cattle included determination of the peak

percent infected erythrocytes (PPE), percent reduction in the packed cell volume (PCV), and the prepatent period (days) determined from the day of challenge-exposure to the onset of infection.

[p. 14, last para.] 1. *Construction, expression in E. coli and characterization of wild type MSP1a and mutants.* A MSP1a (Oklahoma isolate *msp1α* clone per1 [14]) mutant lacking the tandem repeats was constructed by PCR. Oligonucleotide primers RI0R (5'-CCGAATTCCATGTTAGCGGCTAATTGGCGGCAAGAGATGCG-3') (SEQ. ID NO: 5) and MSP1a3BII (5'-CCAGATCTCTTTACGCCGCCGCCTGCGCC-3') (SEQ. ID NO: 6) were designed to amplify the *msp1α* gene lacking 6 amino acids preceding the repeats and the tandem repeats in a 50 µl volume PCR (0.2 µM each primer, 1.5 mM $MgSO_4$, 0.2 mM dNTP, 1X AMV/Tfl reaction buffer, 5u *Tfl* DNA polymerase) employing the Access RT-PCR system (Promega, USA). Reactions were performed in an automated DNA thermal cycler (Eppendorf ~~Mastercycler~~MASTERCYCLER® personal, USA) for 35 cycles. After an initial denaturation step of 30 sec at 94°C, each cycle consisted of a denaturing step of 30 sec at 94°C and an annealing-extension step of 2.5 min at 68°C. The program ended by storing the reactions at 4°C. The primers introduced an ATG initiation codon and *Eco* RI and *Bgl* II restriction sites for cloning into the pFLAG-CTC expression vector (Sigma). The resulting plasmid pAF0R1 was transformed into *E. coli* JM109 and induced for expression of mutant MSP1a as previously reported for MSP1a [14]. For the expression of MSP1a (Oklahoma isolate *msp1α* clone per1 [14]) tandem repeats in *E. coli*, this region was amplified using oligonucleotide primers RNOKBS5 (5'-GAGATCTGCTGATGGCTCGTCAGCGGG-3') (SEQ. ID NO: 7) and RNOKBS3 (5'-GGTCGACCCTGATTGAGACGATGTACTGGCC-3') (SEQ. ID NO: 8). The

PCR was conducted as previously described but with amplification cycles consisting of a denaturing step of 30 sec at 94°C, an annealing step of 30 sec at 58°C and an extension step of 1 min at 68°C. The 5' and 3' amplification primers contained *Bgl* II and *Sal* I restriction sites, respectively, for cloning into pFLC1b [14] for expression in *E. coli* as a fusion peptide to the COOH-terminus of MSP1b (*locus* ß1, Oklahoma isolate). The resulting plasmid pF1bRNO4 was transformed into *E. coli* JM109 and induced for expression of mutant MSP1b>MSP1a-repeats protein as previously reported for MSP1b [14]. All constructs were sequenced at the Core Sequencing Facility, Department of Biochemistry and Molecular Biology, Noble Research Center, Oklahoma State University using ABI Prism dye terminator cycle sequencing protocols developed by Applied Biosystems (Perkin-Elmer Corp., Foster City, CA).

[p. 19, first full para.] Preferably, the inventive vaccine formulation is set to contain about 100 micrograms of recombinant antigens associated to *E. coli* membranes in an oil-based adjuvant such as ~~Xtend~~XTEND® III (Grand Laboratories, Larchwood, IA).